METHOD OF INCREASING BONE VOLUME

TECHNICAL FIELD

The present invention relates to novel methods of increasing bone volume comprising activating the osteoblastic protein kinase C/ intracellular calcium pathways of a subject. This invention further relates to a method of treating or preventing bone disorders wherein activation occurs by the administration of a FP agonist.

BACKGROUND OF THE INVENTION

In osteoporotics an imbalance in the bone remodeling process develops in which bone is resorbed at a rate faster than it is being made. Although this imbalance occurs to some extent in most individuals, both male and female, as they age, it is much more severe and occurs at a younger age in osteoporotics, particularly those who develop the post menopausal form of the condition. Accelerated bone loss may also result from drug administration, such as corticosteroids; prolonged bedrest; disuse of a limb; and microgravity. A consequence of this loss of bone is the complete removal of trabeculae and a deterioration of bone architecture such that the remaining bone is disproportionately decreased in strength.

It is thought that to completely return the bone to normal strength, new trabeculae should be formed to restore architecture and increase bone mass. It is further thought that when the restoration of normal architecture is associated not only with an increase in the strength, but also a return to normal stiffness and shock absorbing capability, the bone is less likely to fracture. Subjects suffering from other bone disorders such as osteoarthritis, Paget's disease, periodontal disease, and fractures may also benefit from treatments that restore bone mass and normal architecture to bone.

There have been many attempts to treat bone disorders with a variety of pharmacologic compounds with the goal being to either slow further bone loss or to produce a net gain in bone mass. For example, there are antiresorptive agents, such as bisphosphonates, which only slow further bone loss. In addition, there are known bone anabolic agents such as PTH and PGE_2. But, neither of these agents builds bone that is substantially similar, i.e. structurally or architecturally, to the type of bone lost.

PTH and PGE_2 are known to stimulate both the cAMP and protein kinase C/ intracellular calcium pathways. In general, it is believed that stimulating the cAMP pathway is necessary, and may be sufficient, to build bone. This belief is based, at least in part, upon observations that PTH analogs that only increase cAMP increase trabecular thickness, while PTH analogs that only increase PKC/Ca++ do not increase bone mass.

This belief is further based on the observation that PGE_2 analogs that selectively stimulate the EP_2 receptor, which is coupled primarily to the cAMP pathway, increase bone volume. PCT Publication WO 98/27976.

In addition to building bone that is not substantially similar to the type of bone lost, these known anabolic agents such as PTH and PGE_2 have several drawbacks which limit their desirability for systemic administration. For example, although prostaglandins are characterized by their activity at a particular prostaglandin receptor, their activity is not limited to any one prostaglandin receptor. Thus, systemic administration of prostaglandins is known to cause side effects such as inflammation, as well as surface irritation, smooth muscle contraction, bronchoconstriction, and vasoconstriction. Systemic administration of non-selective prostaglandin analogs can likewise cause side effects.

Thus, there is a continuing need to develop methods of replacing bone that result in bone that is substantially similar, structurally and architecturally, to the type of bone lost.

SUMMARY OF THE INVENTION

It has been unexpectedly found that agents which primarily activate the osteoblastic protein kinase C/ intracellular calcium pathways significantly improve bone quality over other anabolic agents. Particularly preferred agents are agents that are selective for FP receptors. Particularly preferred agents selective for FP receptors are non-naturally-occurring FP agonist. Particularly preferred non-naturally-occurring FP agonists are selective for the FP receptor over other excitatory prostaglandin receptors in a ratio of at least about 1:10, more preferably at least about 1:20, and most preferably at least about 1:50. Still more preferred non-naturally-occurring FP agonists are selective for the FP receptor over all other prostanoid receptors in a ratio of at least about 1:10, more preferably at least about 1:20, and most preferably at least about 1:50.

It has been further found that agents which primarily activate the osteoblastic protein kinase C/ intracellular calcium pathways increase trabecular number through formation of new trabeculae, increase bone volume and mass while maintaining a normalized bone turnover rate and increase formation at the endosteal surface without removing bone from the existing cortex.

Accordingly, the present invention is directed to methods of increasing bone volume, increasing trabecular number, and treating bone disorders by primarily activating the osteoblastic protein kinase C/ intracellular calcium pathways.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a representative drawing of the protein kinase C/ intracellular Ca^{++} pathways.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is directed to methods of increasing bone volume, methods of increasing trabecular number, and methods of treating bone disorders by primarily activating the ostoeblastic protein kinase C/ intracellular calcium pathways ("PKC/Ca^{++} pathways").

Definitions and Usage of Terms

As used herein, "activates a subject's ostoeblastic protein kinase C /intracellular calcium pathways" refers to the activation of the Gq family of proteins associated with seven transmembrane receptor proteins which initiates the intracellular messenger cascade containing both protein kinase C (PKC) and Ca^{++}.

As used herein, "anabolic agent" means an agent which increases bone.

As used herein, "bone disorder" means the need for bone repair or replacement. Conditions in which the need for bone repair or replacement may arise include: osteoporosis (including post menopausal osteoporosis, male and female senile osteoporosis and corticosteroid induced osteoporosis), osteoarthritis, Paget's disease, osteomalacia, multiple myeloma and other forms of cancer, prolonged bed rest, chronic disuse of a limb, anorexia, microgravity, exogenous and endogenous gonadal insufficiency, bone fracture, non-union, defect, prosthesis implantation and the like.

As used herein, "bone turnover rate" means the amount of bone resorption and formation per unit time measured or estimated using incorporation of fluorescent labels into bone, fluorescent and bright field microscopy, and histomorphometric techniques or by measurement of bone metabolism markers. For example, a subject may resorb and replace (turn over) approximately 3% of its skeleton over a 3 month period. A further description of histomorphometric techniques can be found in Bone Histomorphometry, 1994, by Eriksen et. al., Raven Press.

As used herein, "bone volume" refers to the percentage of the bone occupied by a mineralized matrix. Measurement or estimation of the mineralized matrix volume can be accomplished using histomorphometry, computed tomography, or magnetic resonance imaging. Two dimensional measurements may be used to estimate the three dimensional volume. A further description of histomorphometric techniques can be found in Bone Histomorphometry, 1994, by Eriksen et. al., Raven Press.

As used herein, "excitatory prostaglandin receptor" means prostanoid receptors which cause contraction of smooth muscle or release of internal calcium stores. Such receptors include but are not limited to FP, EP_1, EP_3, TP_1 and TP_2.

As used herein, "FP" is an abbreviation for F prostanoid.

As used herein, "FP agonist" means a compound with affinity for the FP receptor that results in measurable biological activity (including but not limited to an elevation in intracellular calcium or the contraction of smooth muscle) in cells, tissues, or organisms which contain the FP receptor. Whole cell, tissue, and organism assays which demonstrate FP activity of compounds are well known in the art. One particularly useful assay is the R-SAT™ Assay described by Brann, et al. in J. Biomole. Screen, Vol. 1, Number 1, 1996.

As used herein, "FP receptor" means known human FP receptors, their splice variants, and undescribed receptors that preferentially bind $PGF_{2\alpha}$. A human FP receptor is disclosed in PCT Publication WO 95/00551.

As used herein, "measurable" means the biologic effect is both reproducible and significantly different from the baseline variability of the assay.

As used herein, "non-naturally-occurring" means an agent that is not biologically derived in mammals.

As used herein, "osteoblastic" refers to cells of the osteoblast lineage, which includes precursor or progenitor cells, pre-osteoblasts, osteoblasts, bone lining cells and osteocytes.

As used herein, "primarily" means the agent preferentially activates the osteoblastic PKC/Ca^{++} pathways over the cAMP pathway. Preferential activation of the PKC/Ca++ pathways over the cAMP pathway can be measured using a variety of assays. For example, intracellular Ca^{++} concentration can be measured by the use of the calcium indicator Fura-2 assay described in "The molecular Biology of the Cell", edited by Alberts et al., Garland Publishing, 1994, p. 183, and intracellular cAMP can be measured by the assay described in "The principles of bone biology" edited by J Bilezikian et al., Academic Press, 1996, p 1205. To provide a direct comparison of these two pathways, a ratio of activation of 1 is defined as 100% of the maximum activation of the PKC/Ca^{++} pathways of PGE_2 divided by 100% of the activation of the cAMP pathway by PGE_2. The concentrations of PGE_2 required to reach the maximum activation may differ by up to 2 orders of magnitude. Preferential activation would then represent an increase in this ratio above about 1, preferably above about 2, more preferably above about 3.5, and most preferably above about 5, where the pathway activator could either increase the

PKC/Ca^{++} pathway activation (for example to 125% of PGE_2) or decrease the cAMP pathway activation (for example to 75% of PGE_2).

As used herein, "prostaglandin analog" refers to a non-naturally-occurring compound which is structurally similar to a prostaglandin.

As used herein, "prostaglandin receptor" or "prostanoid receptor" means a naturally-occurring protein that binds prostaglandins, which when bound alters the function of a cell. Prostaglandin receptors may be characterized as either excitatory or relaxant. Such receptors include but are not limited to FP, EP_1, EP_2, EP_3, EP_4, DP, IP, TP_1 and TP_2. These receptors are further discussed by Coleman et al., in Pharmacological Reviews, 1994, Volume 6, No. 2, pages 205 - 229.

As used herein, "selective" means having an activation preference for a specific receptor over other receptors which can be quantified based upon whole cell, tissue, or organism assays which demonstrate receptor activity, such as the R-SAT™ Assay disclosed above. A compound's selectivity is determined from a comparison of its EC_{50} (or ED_{50} if using an organism assay) at the relevant receptors. For example, a compound having an EC_{50} of 8nM at the FP receptor and an EC_{50} of 80 nM at the EP_1 receptor has a selectivity ratio for the FP receptor over the EP_1 receptor of 1:10.

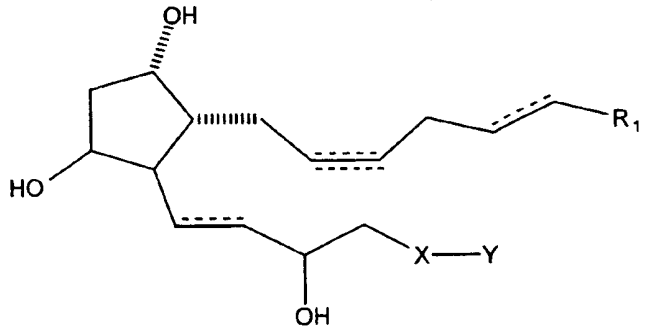
As used herein, "subject" means a living vertebrate animal such as a mammal (especially human) in need of treatment.

As used herein, "trabecular number" refers to the number of individual trabeculae of bone per unit volume of cancellous bone measured or estimated from a two dimensional representation or a three dimensional specimen using histomorphometry, computed tomography, or magnetic resonance imaging.

Compounds

Agents of the present invention primarily activate the ostoeblastic PKC/Ca^{++} pathways. The pathway is shown in FIG. 1. Agents useful in the present invention increase bone volume by primarily activating the ostoeblastic PKC/Ca^{++} pathways. Particularly preferred agents are agents that are selective for FP receptors. Particularly preferred agents selective for FP receptors are non-naturally-occurring FP agonist. Particularly preferred non-naturally-occurring FP agonists are selective for the FP receptor over other excitatory prostaglandin receptors in a ratio of at least about 1:10, more preferably at least about 1:20, and most preferably at least about 1:50. Still more preferred non-naturally-occurring FP agonists are selective for the FP receptor over all other prostanoid receptors in a ratio of at least about 1:10, more preferably at least about 1:20, and most preferably at least about 1:50.

Particularly useful non-naturally-occurring selective FP agonists are prostaglandin analogs. Examples of such compounds are prostaglandin analogs having the following general structure:



wherein:

R_1 is CO_2H, $C(O)NHOH$, CO_2R_2, CH_2OH, $S(O)_2R_2$, $C(O)NHR_2$, $C(O)NHS(O)_2R_2$, or tetrazole; characterized in that R_2 is alkyl, heteroalkyl, carbocyclic aliphatic ring, heterocyclic aliphatic ring, aromatic ring, or heteroaromatic ring;

X is $(CH_2)_n$, where n is 0 to 3, NH, S, or O; and

Y is a cycloalkyl or aromatic moiety, either substituted or unsubstituted.

Prostaglandin analogs of the above structure include: cloprostenol (Estrumate®), fluprostenol (Equimate®), tiaprost, alfaprostol, delprostenate, froxiprost, latanoprost, 13,14-dihydro-16-((3-trifluoromethyl)phenoxy)-16-tetranor prostaglandin $F_1\alpha$, 17-((3-trifluoromethyl)phenyl)-17-trinor-prostaglandin $F_2\alpha$, 13,14-dihydro-18-thienyl-18-dinor prostaglandin $F_1\alpha$ and their analogs.

Other prostaglandin analogs of the present invention include 9-alpha, 11-alpha, 15-alpha-trihydroxy-16-(3-chlorophenoxy)-omega-tetranor-prosta-4-cis-13-trans-dienoic acid and its analogs. Additional prostaglandin analogs are also disclosed in CRC Handbook of Eicosanoids: Prostaglandins and Related Lipids, *Volume I, Chemical and Biochemical Aspects, Part B.* Ed. by Anthony L. Willis, CRC Press (Boca Raton, 1987) Table Four pp. 80-97 (incorporated herein by reference), and references therein.

Methods of Use

The agents described above are useful in increasing bone volume, increasing trabecular number through formation of new trabeculae, increasing bone mass without increasing the bone turnover rate, and increasing formation at the endosteal surface without removing bone from the existing cortex. Additionally, the quality of bone

formed by the administration of these agents is superior to that formed by the administration of other bone anabolic agents, including prostaglandins of the E series. Bone quality refers to the combination of bone matrix (inorganic and organic), bone mass or volume, and bone architecture which impart overall strength and fracture resistance to bone. Accordingly, these agents are further useful in the treatment and prevention of a variety of bone disorders.

The preferred routes of administration for increasing bone volume and treating bone disorders are transdermal and subcutaneous, e.g. injection or pellet. Other preferred routes of administration include oral, sublingual, and intranasal.

The dosage range for systemic administration of the non-naturally-occurring FP agonists of the present invention is from about 0.01 to about 1000 μg/kg body weight per day, preferably from about 0.05 to about 100 μg/kg per body weight per day, most preferably from about 0.1 to about 50 μg/kg body weight per day. Plasma levels are expected to be in the range of about 0.01 to about 500 ng/ml, more preferably from about 0.05 to 100 ng/ml, and most preferably from about 0.1 to 50 ng/ml.

While these dosages are based upon a daily administration rate, weekly or monthly accumulated dosages may also be used to calculate the clinical requirements. The non-naturally-occurring FP agonists of the present invention may be administered, based on a weekly dosage, more frequently than once daily. The non-naturally-occurring FP agonists of the present invention may also be administered, based on a weekly dosage, less frequently than once daily. Hence, the weekly dosage may be divided into 3, 4, 5, 6, or 7 daily dosages, preferably 5, 6, or 7 daily dosages.

Dosages may be varied based on the patient being treated, the condition being treated, the severity of the condition being treated, and the route of administration to achieve the desired effect.

It has been further discovered that prolonged delivery (also referred to as "prolonged administration") of the non-naturally-occurring FP agonist unexpectedly results in improved dose separation between side effects and the desired bone effect. That is, as used herein, "prolonged delivery" or "prolonged administration" means that the total daily dosage is delivered into the subject's circulation over a period of at least about 6 hours and up to 24 hours. Preferred prolonged delivery periods are for at least about 12 hours and up to 24 hours. Examples of prolonged delivery include administration of the non-naturally-occurring FP agonist via a transdermal patch or a subcutaneous pump that delivers the total daily dosage over a twenty-four hour period.

It is believed that the flattening of the plasma concentration curve resulting from prolonged delivery mitigates side effects while maintaining bone efficacy. It is further

believed that the administration of non-naturally-occurring FP agonists with extended half-lives will likewise result in a flattening of the plasma concentration curve without prolonging the administration.

The following non-limiting examples serve to further illustrate the use of the agents of the present invention.

Example I

The FP agonist, fluprostenol, is administered to a 65 year old woman who has decreased bone mass and has been diagnosed with osteoporosis by her physician. She is treated daily with a transdermal patch that delivers 10 μg/kg fluprostenol over a 24 hour period. This treatment is continued for 24 months, at which time, vertebral bone mass is substantially increased compared to her vertebral bone mass at the onset of therapy as measured by dual energy X-ray absorptiometry (DXA).

Example II

The FP agonist, fluprostenol, is administered to a 63 year old woman who has decreased bone mass and has been diagnosed with osteoporosis by her physician. She is treated with an implantable subcutaneous pump that delivers 10 μg/kg fluprostenol over a 24 hour period. This treatment is continued for 12 months, at which time, vertebral bone mass is substantially increased compared to her vertebral bone mass at the onset of therapy as measured by dual energy X-ray absorptiometry (DXA).

Pharmaceutical Formulations

Pharmaceutical formulations of the present invention comprise a safe and effective amount of the non-naturally-occurring FP agonist and a pharmaceutically acceptable carrier.

The phrase "safe and effective amount", as used herein means an amount of a compound or composition high enough to significantly positively modify the symptoms and/or condition to be treated, but low enough to avoid serious side effects (at a reasonable benefit/risk ratio), within the scope of sound medical judgment. The safe and effective amount of an agent for use in the method of the invention herein will vary with the particular condition being treated, the age and physical condition of the patient being treated, the severity of the condition, the duration of the treatment, the nature of concurrent therapy, the particular agent being employed, the particular pharmaceutically-acceptable excipients utilized, and like factors within the knowledge and expertise of the attending physician.

In addition to the compound, the compositions of the subject invention contain a pharmaceutically-acceptable carrier. The term "pharmaceutically-acceptable carrier", as used herein, means one or more compatible solid or liquid filler diluents or encapsulating substances which are suitable for administration to a subject. The term "compatible", as used herein, means that the components of the composition are capable of being commingled with the compound, and with each other, in a manner such that there is no interaction which would substantially reduce the pharmaceutical efficacy of the composition under ordinary use situations. Pharmaceutically-acceptable carriers must, of course, be of sufficiently high purity and sufficiently low toxicity to render them suitable for administration to the subject being treated.

Some examples of substances which can serve as pharmaceutically-acceptable carriers or components thereof are sugars, such as lactose, glucose and sucrose; starches, such as cornstarch and potato starch; cellulose and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose, cellulose acetate; powdered tragacanth; malt; gelatin; talc; solid lubricants, such as stearic acid, magnesium stearate; calcium sulfate; vegetable oils, such as peanut oil, cottonseed oil, sesame oil, olive oil, corn oil and oil of theobroma; polyols such as propylene glycol, glycerin, sorbitol, mannitol, and polyethylene glycol; alginic acid; emulsifiers, such as the Tweens®; wetting agents such as sodium lauryl sulfate; coloring agents; flavoring agents, excipients; tableting agents; stabilizers; antioxidants; preservatives; pyrogen-free water; isotonic saline; and phosphate buffer solutions.

The choice of a pharmaceutically-acceptable carrier to be used in conjunction with a compound is basically determined by the way the compound is to be administered. The non-naturally-occurring FP agonist of the present invention may be administered systemically, including transdermally, orally and/or parenterally, including subcutaneous or intravenous injection, and/or intranasally.

The appropriate amount of the agent, preferably non-naturally-occurring FP agonist, to be used may be determined by routine experimentation with animal models. Such a model includes, but is not limited to, the intact and ovariectomized rat models of osteoporosis, the ferret, canine, and non human primate models of osteoporosis, as well as disuse models of osteoporosis.

A preferred method of administering non-naturally-occurring FP agonists is via transdermal delivery. Preferred transdermal dosage forms include transdermal patches, creams, ointments, gels and the like. Another preferred method of administering non-naturally-occurring FP agonists is via subcutaneous injection in a unit dosage form.

Preferred unit dosage forms for injection include sterile solutions of water, physiological saline, or mixtures thereof. The pH of said solutions should be adjusted to about 7.4.

Other preferred dose forms include nasal, rectal, sublingual, and oral. Suitable carriers for injection or surgical implants include hydrogels, controlled- or sustained-release devises, polylactic acid, and collagen matrices. Implant devices may be coated with the non-naturally-occurring FP agonist. The non-naturally-occurring prostaglandin FP agonist may be dissolved in a buffer and may be mixed with a collagen gel which is then coated onto the porous end of the implant device.

Preferred oral forms include, for example liposomes, lipid emulsions, proteinaceous cages and pharmaceutically-acceptable excipients.

The term "pharmaceutically-acceptable excipients" as used herein includes any physiologically inert, pharmacologically inactive material known to one skilled in the art, which is compatible with the physical and chemical characteristics of the particular active ingredient selected for use. Pharmaceutically-acceptable excipients include, but are not limited to, polymers, resins, plasticizers, fillers, lubricants, binders, disintegrants, solvents, co-solvents, buffer systems, surfactants, preservatives, sweetening agents, flavoring agents, pharmaceutical grade dyes and pigments.

The following non-limiting examples illustrate formulations of the subject invention.

Example III

Pharmaceutical formulations (compositions) in the form of tablets are prepared by conventional methods, such as mixing and direct compaction, formulated as follows

Ingredient	Quantity (mg per tablet)
Fluprostenol	5
Microcystalline Cellulose	100
Sodium Starch Glycollate	30
Magnesium Stearate	3

The above tablet administered orally once daily for six months substantially increases bone volume of a patient afflicted with Osteoporosis.

Example IV

A pharmaceutical composition in liquid form is prepared by conventional methods, formulated as follows:

Ingredient	Quantity
Cloprostenol	5mg

Phosphate buffered physiologic saline	10 ml
Methyl paraben	0.05 ml

1.0 ml of the above composition administered subcutaneously once daily for six months substantially increases bone volume of a patient afflicted with osteoporosis.

While particular embodiments of the subject invention have been described, it would be obvious to those skilled in the art that various changes and modifications to the compositions disclosed herein can be made without departing from the spirit and scope of the invention.

What is claimed is:

1. The use of an agent which primarily activates a subject's osteoblastic protein kinase C/ intracellular calcium pathways in the manufacture of a medicament for increasing bone volume in the subject.

2. The use of an agent which primarily activates a subject's osteoblastic protein kinase C/ intracellular calcium pathways in the manufacture of a medicament for increasing trabecular number in a subject.

3. The use of an agent which primarily activates subject's osteoblastic protein kinase C/ intracellular calcium pathways in the manufacture of a medicament for treating a bone disorder in a subject.

4. The use of Claim 1, 2, or 3 characterized in that the agent is a non-naturally-occurring selective FP agonist.

5. The use of Claim 4 characterized in that the non-naturally-occurring FP agonist is selective for the FP receptor over other excitatory prostaglandin receptors in a ratio of at least 1:10.

6. The use of Claim 5 characterized in that the non-naturally-occurring FP agonist is further selective for the FP receptor over all other prostanoid receptors in a ratio of at least 1:10.

7. The use of Claim 6 characterized in that the non-naturally-occurring FP agonist is further selective for the FP receptor over other excitatory prostaglandin receptors in a ratio of at least 1:20.

8. The use of Claim 7 characterized in that the non-naturally-occurring FP agonist is further selective for the FP receptor over all other prostanoid receptors in a ratio of at least 1:20.

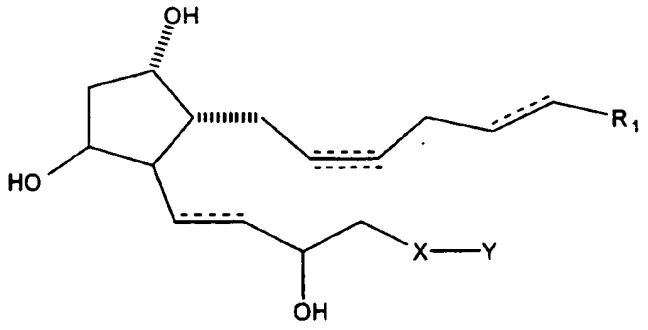
9. The use of Claim 8 characterized in that the non-naturally-occurring FP agonist is further selective for the FP receptor over other excitatory prostaglandin receptors in a ratio of at least 1:50.

10. The use of Claim 9 characterized in that the non-naturally-occurring FP agonist is further selective for the FP receptor over all other prostanoid receptors in a ratio of at least 1:50.

11. The use of Claim 10 characterized in that the non-naturally-occurring FP agonist is a prostaglandin analog.

12. The use of Claim 11 characterized in that the medicament allows transdermal delivery of the non-naturally-occurring FP agonist.

13. The use of Claim 11 characterized in that the prostaglandin analog has the general formula:



characterized in that:

R_1 is CO_2H, $C(O)NHOH$, CO_2R_2, CH_2OH, $S(O)_2R_2$, $C(O)NHR_2$, $C(O)NHS(O)_2R_2$, or tetrazole; characterized in that R_2 is alkyl, heteroalkyl,

carbocyclic aliphatic ring, heterocyclic aliphatic ring, aromatic ring, or heteroaromatic ring;

X is $(CH_2)_n$, where n is 0 to 3, NH, S, or O; and

Y is a cycloalkyl or aromatic moiety, either substituted or unsubstituted.

14. The use of Claim 11 characterized in that the prostaglandin analog is fluprostenol.

15. The use of Claim 14 characterized in that the medicament allows transdermal delivery of the fluprostenol.

16. The use of Claim 8 characterized in that the non-naturally-occurring FP agonist is selected from the group consisting of cloprostenol (Estrumate®), fluprostenol (Equimate®), tiaprost, alfaprostol, delprostenate, froxiprost, 9-alpha, 11-alpha, 15-alpha-trihydroxy-16-(3-chlorophenoxy)-omega-tetranor-prosta-4-cis-13-trans-dienoic acid, 17-((3-trifluorormethyl)phenyl-17-trinor-prostaglandin $F_2\alpha$, 13,14-dihydro-18-thienyl-18-dinor prostaglandin $F_1\alpha$, 13,14-dihydro-16-((3-trifluoromethyl)phenoxy)-16-tetranor prostaglandin $F_1\alpha$, latanoprost, and their analogs.

17. The use of Claim 3 characterized in that the bone disorder is selected from the group consisting of: osteoporosis, osteoarthritis, Paget's disease, osteomalacia, and bone fracture.

18. The use of Claim 3 characterized in that the bone disorder is osteoporosis.

19. The use of Claim 18 characterized in that the non-naturally-occurring FP agonist is fluprostenol.

20. The use of Claim 18 characterized in that the bone disorder is post-menopausal osteoporosis.

21. The use of Claim 1, 2, or 3 characterized in that the medicament allows for prolonged administration of the non-naturally-occurring FP agonist.

22. The use of Claim 20 characterized in that the medicament allows for delivery of the non-naturally-occurring FP agonist over a period of at least twelve hours.

23. The use of Claim 1, 2, or 3 characterized in that the medicament does not substantially increase the subject's bone turnover.

INTERNATIONAL SEARCH REPORT

Int onal Application No
PCT/US 98/18337

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K31/557

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
X	WO 93 15767 A (MERCK & CO. INC.) 19 August 1993 see claims 1 & 2 ---	1-3,17, 18,21-23
X	WO 97 31640 A (PFIZER INC.) 4 September 1997 see page 1, line 21 see page 29, line 27 - page 31, line 31 ---	1-4,17, 18,20
X	RAISZ L G ET AL: "Effect of alterations in the cyclopentane ring on bone resorptive activity of prostaglandin" PROSTAGLANDINS, vol. 37, no. 2, 1989, pages 229-36, XP002091503 see abstract and description page 234 --- -/--	1-4

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
29 January 1999	18/02/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Mair, J

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Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

In[illegible]al Application No
PCT/US 98/18337

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	MA, Y.F. ET AL: "Effects of prostaglandin E2 and F2alpha on the skeleton of osteopenic ovarectomized rats" BONE, vol. 17, no. 6, December 1995, pages 549-554, XP002091504 see page 553, right-hand column, line 19 - page 554, left-hand column, line 20	1-3

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

national application No.
PCT/US 98/ 18337

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 1-13,16-23 partially
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
See FURTHER INFORMATION SHEET PCT/ISA/210

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Claims Nos.: 1-13, 16-23 (partially)

In view of the large number of compounds which are theoretically defined by the open-ended definition of claims 1-3 and 16 and the Markush formula of claim 13 the search has had to be restricted on economic grounds to the specifically claimed compounds and the general concept of the application (see guidelines, Chapter III, paragraph 2.3).

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 98/18337

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9315767 A	19-08-1993	AU 3607293 A	03-09-1993
WO 9731640 A	04-09-1997	AU 1039897 A	16-09-1997
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		HR 970118 A	30-04-1998

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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : A61K 31/557

A1

(11) International Publication Number: **WO 99/12550**

(43) International Publication Date: 18 March 1999 (18.03.99)

(21) International Application Number: PCT/US98/18337

(22) International Filing Date: 4 September 1998 (04.09.98)

(30) Priority Data:
60/058,218 9 September 1997 (09.09.97) US

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(81) Designated States: AL, AM, AT, AT (Utility model), AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, CZ (Utility model), DE, DE (Utility model), DK, DK (Utility model), EE, EE (Utility model), ES, FI, FI (Utility model), GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK (Utility model), SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published

With international search report.

Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: METHOD OF INCREASING BONE VOLUME

(57) Abstract

The present invention relates to novel methods of increasing bone volume comprising activating the osteoblastic protein kinase C/intracellular calcium pathways of a subject. This invention further relates to a method of treating or preventing bone disorders wherein activation occurs by the administration of a FP agonist.

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